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CERTIFICATE

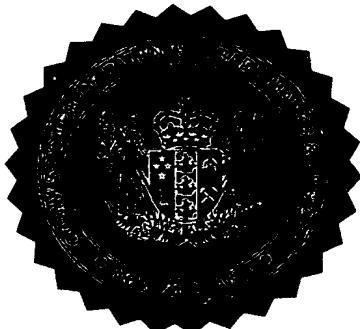
This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 12 May 2000 with an application for Letters Patent number 504523 made by DIATRANZ LIMITED.

Dated 1 February 2001.

PRIORITY DOCUMENT
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Neville Harris
Commissioner of Patents



504523

NEW ZEALAND
PATENTS ACT, 1953

PROVISIONAL SPECIFICATION

"Treatment of Xenotransplanted Patients."

We, DIATRANZ LIMITED, a company duly incorporated under the laws of New Zealand of 19 Laureston Avenue, Papatoetoe, Auckland, New Zealand, do hereby declare this invention to be described in the following statement:

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The present invention relates to improvements in and/or relating to the treatment of diabetes using suitable insulin producing porcine islet preparations in patients subjected to a cholesterol drug lowering regime or a "statin" family drug regime.

The present invention relates to the treatment of a mammalian patient suffering from diabetes (including humans) which involves the transplantation into the mammal of viable encapsulated porcine islets capable of producing insulin within its host.

Type 1 (insulin-dependent) diabetes mellitus is a common endocrine disorder that results in substantial morbidity and mortality, and leads to considerable financial costs to individual patients and healthcare systems.

Treatment with insulin, while life-saving, often does not provide sufficient control of blood glucose to prevent the feared complications of the disease, which has provided the impetus for intensive research into better methods of sustaining normoglycaemia.

Among the newer treatment strategies that have been proposed, transplantation of pancreatic β islet cells, obtained either from other humans or animals, has received the most attention worldwide. This is because transplantation can restore not only the insulin-secreting unit, but also the precise fine tuning of insulin release in response to multiple neural and humoral signals arising within and beyond the islets of Langerhans.

Human islet cell transplantation is limited by the shortage of human islet tissue. The use of pig islet cells is currently viewed as the most promising alternative since:

- (a) the supply of pig cells can be readily expanded by optimising the supply of donor animals;
- (b) pig and human insulin have close structural similarities; and
- (c) physiological glucose levels in pigs are similar to those in humans.

The rationale for this treatment approach (termed 'xenotransplantation') is that the implanted pig islets have the potential to mimic the normal physiological insulin response in type 1 diabetics such that near-normal blood glucose levels may be achievable without insulin administration or with a reduced requirement for it. As a consequence, long-term diabetes complications may be prevented and patients should experience less hypoglycaemia than they do with the currently recommended 'intensive' insulin regimens.

In a first aspect the invention consists in a method for treatment of a mammalian patient suffering from diabetes which comprises:

1. extracting pancreatic β islet cells from piglets at or near full term gestation;
2. treating said islets with nicotinamide,
3. encapsulating said islets in a biocompatible material which will allow *in vivo* glucose movement to and insulin movement from the islets, and
4. injecting or otherwise implanting the encapsulated islet cells of step (c) so as to transplant into said mammalian patient an effective amount of viable

piglet islet cells capable of producing insulin in the patient, and

5. (optionally) administering nicotinamide to said mammalian patient at least subsequent to transplantation; and
6. (optionally) administering a casein-free as herein defined diet to said mammalian patient, the procedure being **characterised in that the patient has prior to, during or after the step (d) been subjected to a cholesterol lowering drug regime.**

Preferably said drug is of the "statin" family.

Preferably said drug is pravastatin.

As used herein "administering" includes self administering.

Preferably said piglets at or near full term gestation from which the pancreatic β islet cells are extracted are at from -20 to +10 days full term gestation.

Preferably said piglets are at from -7 to +10 days full term gestation.

Preferably said encapsulation is with an alginate material (preferably sodium alginate) (whether after pre-coating or not with some other material).

Preferably said alginate (preferably sodium alginate) is in ultra pure form.

Preferably each islet or grouping of islets is entrapped in an *in vivo* insulin and glucose porous biocompatible alginate or alginate like surround.

Preferably such coating prevents, once implanted, direct tissue contact with said islets and/or any pre-coating matrix which itself has the requisite porosity once implanted.

Preferably each encapsulation involves presenting islets and a suitable alginate solution into a source of compatible cations thereby to entrap the islets in a cation - alginate gel.

Preferably said cation alginate gel is calcium-alginate gel.

Preferably said alginate used in the solution is sodium alginate.

Preferably the islets and sodium alginate solution (preferably 1.6% w/w) is presented as a droplet (eg. through a droplet generating needle) into a bath of suitable cations (eg. gelating cations such as calcium chloride).

Preferably the gel encased islets are coated with a positively charged material and thereafter optionally are provided with an outer coat of a suitable alginate.

Preferably said positive charging material is poly-L-ornithine.

Preferably the gel entrapping the islets within the outer coating is then liquified.

Preferably said liquification is by the addition of sodium citrate.

Preferably said capsules contain a plurality of islet cells (preferably about three) and preferably have a diameter of from about 300 to 400 microns.

After liquification of the alginate entrapping the islets, the "capsules" are washed, and again coated with alginate which neutralizes any residual charge on the poly-L-

ornithine coating and prevents direct contact of the poly-L-ornithine with tissues when the entire capsule is transplanted.

Preferably the alginate production process has involved the following steps:

Seaweed harvest → Washing → Alginate extraction → Filtration (preferably a 0.2 μm filter) → Precipitation → Drying.

Preferably the ultrapure alginate is Kelco LV produced by Monsanto-Kelco, US and has the following specifications

1. Viscosity: 2% - 100-300 cps (Brookfield 25 °C, speed 3,60 rpm)
2. pH: 6.4-8.0
3. Protein content <0.5%
4. Filtration: through 0.2 μm
5. Chemical analysis:

Ca: <100 ppm	Mg <40 ppm	Mn: <10 ppm
Cu: <40 ppm	Zn: <40 ppm	Sr: <40 ppm
Fe: <60 ppm	Pb: <50 ppm	As: <100 ppb
Hg: <40 ppb	Si: <10 ppm	
6. Endotoxin level - measured by LAL test (at University of Perugia): 39 EU/g
[NB. Any level below 100 EU/g in this test is considered endotoxin-free].

7. Molecular weight: 120,000 - 190,000 kD
8. Mannuronic acid (M) content: M fraction (F_m) 61%
9. Guluronic acid (G) content: G fraction (F_G) 39%

Preferably the filtration has been with a multiple filtration process employing positively charged filters that remove any lipopolysaccharide content.

Preferably said pancreatic β islet cells at some stage after extraction from the piglets and prior to encapsulation are exposed to IgF₁.

Preferably said exposure to IgF₁ is greater for those cells from piglets furthest from full term gestation but preferably there is exposure to IgF₁ for all cells extracted irrespective of their relationship to full term gestation.

Preferably said extraction involves the use of a trauma protecting agent for the islet cells during the isolation and/or preparation thereof for encapsulation.

Preferably said agent is a trauma protecting agent selected from suitable anaesthetic agents such as, for example, lignocaine.

Preferably the mammalian patient is administered nicotinamide prior to transplantation.

Preferably the casein-free as herein defined diet is administered to the mammalian patient at least after transplantation.

Preferably a casein-free as herein defined diet is administered prior to

transplantation.

“Casein-free” as used herein refers to milk which does not contain a diabetogenic factor, particularly to milk containing no variant of β -casein which stimulates diabetogenic activity in humans. With reference to International PCT Application WO 96/14577, a non-diabetogenic variant for example, may be the A2 variant of β -casein. The full contents of PCT/NZ95/00114 (WO 96/14577) and PCT/NZ96/00039 (WO 96/36239) are here included by way of reference.

The major advantage of porcine islet cell transplantation over human islet cell transplantation is that the islet cell source can be readily expanded, and the biosafety of the cells can be thoroughly explored prior to transplantation. From a practical viewpoint, pancreas removal and islet cell isolation can be performed expeditiously in an ideal environment.

Important considerations relevant to the use of porcine islet cells in transplantation approaches for type 1 diabetes include the following:

- The structural and biological similarities of porcine and human insulin
- The fact that porcine insulin has been used to treat diabetes for several decades (and has only been replaced by human sequence insulin relatively recently); and
- The similarity of physiological glucose levels in pigs and humans. (Weir & Bonner-Weir 1997). This effectively means that pig islet cells can be expected to react similarly to their human counterparts in maintaining equivalent blood glucose concentrations.

1. *The nature of the disease causing diabetes:* successful long-term allotransplantation of human islets can be achieved in over 80% of patients when the disease is caused by non-immune processes. In contrast, even islets obtained from a non-diabetic twin cannot reverse autoimmune diabetes long-term in the diabetic twin member. This emphasises the critical role of autoimmunity in the failure of islet transplantation. This observation has been validated in allotransplantation of rodents with diabetes caused by autoimmunity as compared with diabetes due to pancreatectomy or chemical β cell destruction. No large animal model of autoimmune diabetes exists.

It is possible that the use of islets from different species (xenotransplantation) could avoid autoimmune destruction of transplanted islets, as the immune process of xenotransplant rejection is different to that of allotransplant rejection, but this is entirely hypothetical in humans.

2. *The viability of the islets:*

The processes by which islets are purified prior to transplantation are traumatic to these highly specialised tissues. Such trauma can induce necrosis or apoptosis – the latter being quite delayed.

Further trauma may result from encapsulation. Processes used by us in both the preparation of islets and their encapsulation have been optimised to ensure minimal damage to the islets. Such procedures have ensured zero warm ischaemia (compared with hours with most human islet preparations), have involved the use of nicotinamide to enhance successful *in vitro* explantation, have involved minimal incubation time with collagenase, have involved swift non-traumatic encapsulation technology, etc. Our preferred preparation preferably uses neonatal (7-day old) islets which is crucial in both limiting islet trauma during purification, and assuring sufficient maturation of the islets for stimulated insulin production to occur.

3. *Drugs used in the recipient:* transplantation does not require and avoids the need for cytotoxic agents to suppress the immune system. Such agents are able to enter the alginate microcapsule and cause islet toxicity, as well as causing systemic toxicity. Instead, nicotinamide and a special diet are used (for rationale, see section 1.4 below).

The transplantation procedures of our earlier patent specification have the ability over a period prior to rejection of providing porcine insulin. In this respect, we ourselves conducted clinical trials.

Four type 1 diabetic adolescents received 10,000 free islets/kg bodyweight by intraperitoneal injection. The islets were located from term piglets using the standard collagenase digestion, purification and culture techniques described in section 3.2. All four recipients received oral nicotinamide (1.5 g/day) and a casein-free as herein defined diet both pre- and post-transplantation. A prompt reduction in insulin requirements, which was not clearly dose-related, was noted in the first week after transplantation. The reduction in insulin dosage range from 21 to 32%, and the response lasted for up to 14 weeks. However, insulin doses subsequently returned to their previous levels.

The most likely reason for the transplant failure in these patients was chronic rejection. However, no adverse effects were noted.

We have now shown alginate-encapsulated porcine islet cell transplants in two human diabetic patients, prolonged functioning of the transplants. The islets were transplanted by intraperitoneal injection, one patient receiving 15,000 IEQ/kg (total 1,300,000 islets) and the other 10,000 IEQ/kg (total 930,000 islets). Both patients were treated pre- and post-transplantation with oral nicotinamide and a soy-based/casein-free as herein defined diet.

The preferred procedure as shown in Figure 1 was used for the preparation, the encapsulation being as aforesaid. Islet cells of -7 days to +10 days full gestation were used.

DATED THIS 12th DAY OF May 2000
AJ PARK
PER *J. Kelley*
AGENTS FOR THE APPLICANT

504523

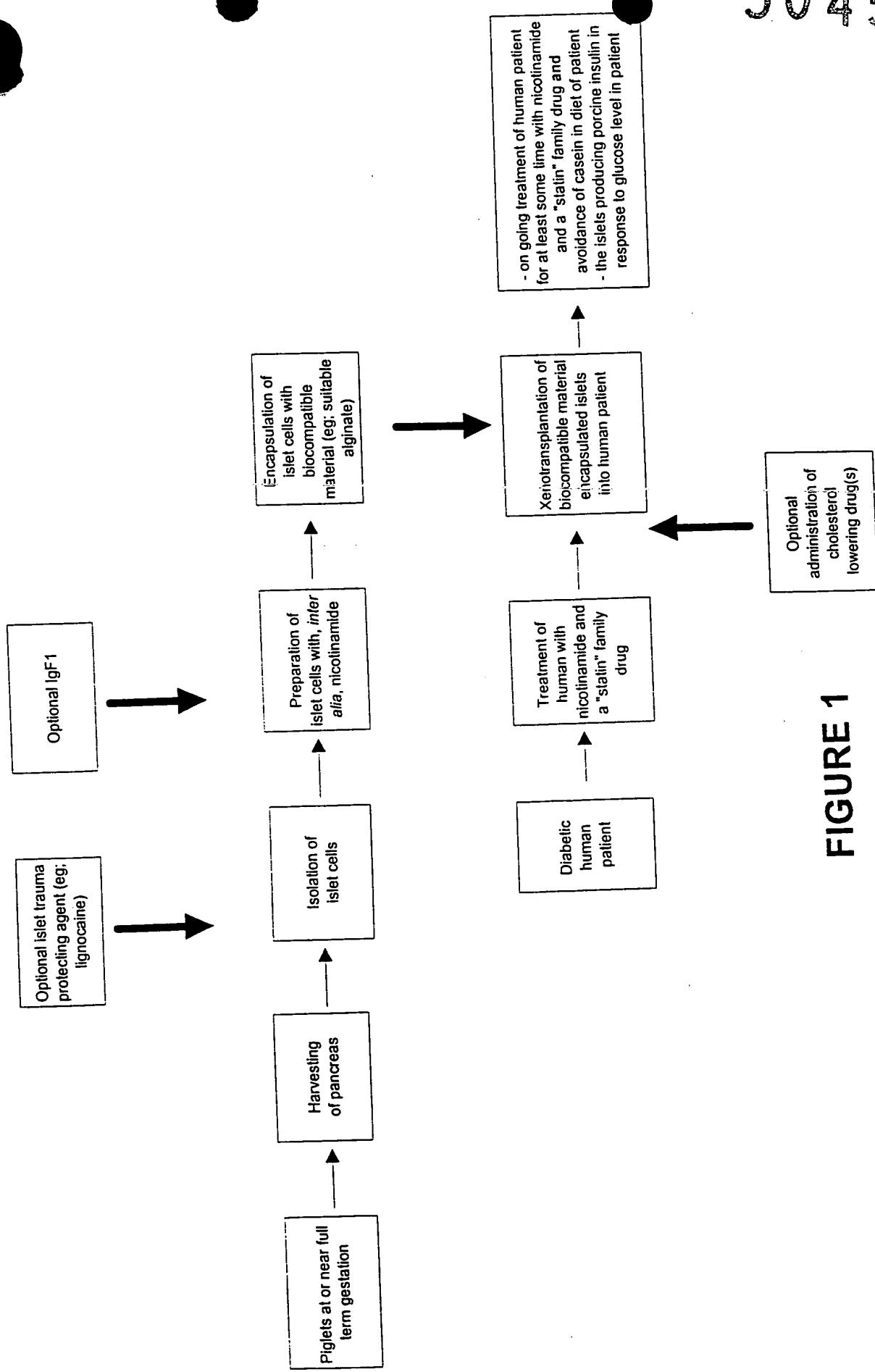


FIGURE 1

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